

AD-A253 610



ON PAGE

Form Approved
GMS No. 0704-0188

go 1 hour per response, including the time for reviewing instructions, searching existing data sources, collection of information, sending comments regarding this burden estimate or any other aspect of this collection of information, including suggestions for reducing the burden, to Washington Headquarters Services, Directorate for Information Operations and Reports, 1215 Jefferson Avenue, Suite 1204, Washington, DC 20540-6001.

1. AGENCY USE ONLY (Leave blank)		2. REPORT DATE 30 JUNE 1992		3. REPORT TYPE AND DATES COVERED Annual Technical/July 1991-June 1992	
4. TITLE AND SUBTITLE Combined Effects of Hyperbaric Oxygen and Antimicrobials In A Model of Gas Gangrene				5. FUNDING NUMBERS AFOSR-90-0317 61102F 2312 A6	
6. AUTHOR(S) MUHVICH KH, ANDERSON LH, MEHM WJ					
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) Armed Forces Institute of Pathology Division of Altitude and Hyperbaric Physiology CPW-P Washington, D.C. 20306-6000				8. PERFORMING ORGANIZATION REPORT NUMBER AFIP/UBFO	
9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES) Air Force Office of Scientific Research Building 410 Bolling AFB, DC 20332				10. SPONSORING/MONITORING AGENCY REPORT NUMBER NOT KNOWN	
11. SUPPLEMENTARY NOTES None					
12a. DISTRIBUTION/AVAILABILITY STATEMENT DOD Approved for public release; distribution unlimited.				12b. DISTRIBUTION CODE	
13. ABSTRACT (Maximum 200 words) <p>The efficacy of hyperbaric oxygen (HBO) alone and in combination with several antimicrobial agents was evaluated in a lethal model of gas gangrene in mice. Myonecrosis was induced by injecting -10/9 washed <i>C. perfringens</i> into the right upper thigh muscle of mice. Immediately following bacterial inoculation, penicillin, imipenem, clindamycin or metronidazole were administered via, intraperitoneal injection. HBO treatments [PO₂ = 301 kPa for 90 min, B.I.D. X days] began immediately after antimicrobial injections. Survival of mice was monitored for 72 h. Mice treated with clindamycin or metronidazole survived significantly longer than mice treated with penicillin G or imipenem (P = 0.05). HBO alone did not prolong time-to-death in control animals as compared to air. Also, HBO did not potentiate the efficacies of any of the antimicrobials tested in this model.</p>					
14. SUBJECT TERMS Hyperbaric Oxygen, Clostridial myonecrosis, alpha toxin, antimicrobials				15. NUMBER OF PAGES	
				16. PRICE CODE	
17. SECURITY CLASSIFICATION OF REPORT Unclassified	18. SECURITY CLASSIFICATION OF THIS PAGE SAR	19. SECURITY CLASSIFICATION OF ABSTRACT SAR	20. LIMITATION OF ABSTRACT SAR		

540-01-280-5500

(0 2 JUL 1992)

Standard Form 298 (Rev. 2-89)
Prescribed by ANSI Std. Z39-18

ABSTRACT

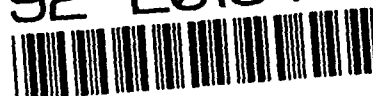
13. The efficacy of hyperbaric oxygen (HBO) alone and in combination with several antimicrobial agents was evaluated in a lethal model of gas gangrene in mice. Myonecrosis was induced by injecting $>10^9$ washed C. perfringens into the right upper thigh muscle of mice. Immediately following bacterial inoculation, penicillin, imipenem, clindamycin or metronidazole were administered via intraperitoneal injection. HBO treatments [$PO_2 = 301$ kPa for 90 min, B.I.D. X 3 days] began immediately after antimicrobial injections. Survival of mice was monitored for 72 h. Mice treated with clindamycin or metronidazole survived significantly longer than mice treated with penicillin G or imipenem ($P < 0.05$). HBO alone did not prolong time-to-death in control animals as compared to air. Also, HBO did not potentiate the efficacies of any of the antimicrobials tested in this model.

Accession For	
NTIS GRA&I	<input checked="" type="checkbox"/>
DTIC TAB	<input type="checkbox"/>
Unannounced	<input type="checkbox"/>
Justification	
By _____	
Distribution/	
Availability Codes	
Dist	Avail and/or Special
A-1	

DTIC QUALITY INSPECTED 2

92 7 27 064

92-20154



INTRODUCTION

1. Objectives

This study was designed to characterize the effects of hyperbaric oxygen (HBO) on four different antimicrobial agents, including the drug of choice penicillin, in a mouse model of clostridial myonecrosis (gas gangrene). To this end, a highly reproducible mouse model of gas gangrene was treated with penicillin, clindamycin, imipenem, or metronidazole with or without HBO. The specific objectives of this study were as follows:

- a) to determine if an intensive HBO treatment protocol would prolong time-to-death in mice not treated with antibiotics.
- b) to characterize the effects of HBO in combination with the above mentioned antibiotics.
- c) to determine if there are any differences in the efficacies of the drugs used alone to treat gas gangrene in this model.

It was the intention of the original protocol to determine a dose response curve for oxygen using the most efficacious antimicrobial agent. However, HBO had no effect alone or in combination with the antibiotics in prolonging time-to-death in this study. Therefore, a dose-response curve for oxygen could not be accomplished.

2. Findings

A cumulative, substantive and comprehensive statement and discussion of research background, rationale, material, methods and scientific significance can be found in the appendices. The major findings of this study are as follows:

- 1) HBO did not prolong time-to-death in control animals as compared to air.
- 2) HBO did not potentiate the efficacies of penicillin, imipenem, clindamycin, or metronidazole.
- 3) All antimicrobials significantly prolonged time-to-death as compared to untreated controls.
- 4) Mice treated with clindamycin or metronidazole survived significantly longer than mice treated with penicillin G or imipenem.

3. Presentations and Publications

A portion of this study will be presented at the 32nd Interscience Conference on Antimicrobial Agents and Chemotherapy to be held in October 1992 in Anaheim, California. The paper to be presented will be published as abstract no. 521 in the Program and Abstracts for the ICAAC meeting.

4. Appendices

Appendix A - Abstract no. 521 to be presented at the 32nd Interscience Conference on Antimicrobial Agents and Chemotherapy to be held in October, 1992.

Appendix B - Draft of a research paper which was submitted to the Journal of Hyperbaric Medicine

APPENDIX A

Evaluation of Antimicrobials Combined
with Hyperbaric Oxygen in a Mouse
Model of Clostridial Myonecrosis.

L.H. ANDERSON*, K.H. MUHVICH, and W.J. MEHM. Armed
Forces Institute of Pathology, Washington, DC.

Antimicrobial chemotherapy and hyperbaric oxygen (HBO) are adjuncts to surgical debridement in human cases of clostridial myonecrosis. The purpose of this study was to determine if HBO alters the efficacy of selected antimicrobials in a lethal mouse model of Clostridium perfringens myonecrosis. Myonecrosis was induced by injecting $>10^9$ washed C. perfringens into the right upper thigh muscle of each mouse. Antimicrobials (penicillin G, imipenem, clindamycin or metronidazole) were injected I.P. within 30 minutes of bacterial injection. HBO treatments [$PO_2 = 301$ kPa for 90 min, B.I.D. x 3 days] began immediately after antimicrobial injections. Survival of mice was monitored for 72 h. Mice treated with clindamycin or metronidazole survived significantly longer than mice treated with penicillin G or imipenem ($p < 0.05$). HBO alone did not prolong time-to-death in control animals as compared to air. Also, HBO did not potentiate the efficacies of any of the antimicrobials tested in this model.

APPENDIX B

**Evaluation of Antimicrobials combined with Hyperbaric Oxygen in a
Mouse Model of Clostridial Myonecrosis**

K. H. MUHVICH, PhD, L.H. ANDERSON, MS, MT(ASCP) and W. J. MEHM,
PhD*

Division of Altitude and Hyperbaric Physiology, Armed Forces
Institute of Pathology, Washington, DC

*Author to whom correspondence should be addressed.

Running Head: Role of Antibiotics and HBO in Gas Gangrene

Support: This study was supported by grant 90-0317 from the Air
Force Office of Scientific Research. K. H. MUHVICH was supported
by the Callender-Binford Fellowship in Pathology sponsored by the
American Registry of Pathology, Washington, D.C. 20306-6000.

Military Status: Both L. H. ANDERSON and W. J. MEHM are Majors in
the Biomedical Sciences Corps of the United States Air Force.

Disclaimer: The opinions or assertions contained herein are the
private views of the authors and are not to be construed as
official or as reflecting the views of the Department of the
Army, the Department of the Air Force, or the Department of
Defense.

ABSTRACT

The efficacy of hyperbaric oxygen (HBO) alone and in combination with several antimicrobial agents was evaluated in a lethal model of gas gangrene in mice. Intraperitoneal administration of penicillin, imipenem, clindamycin, or metronidazole immediately followed inoculation of $>10^9$ CFU of Clostridium perfringens type A in mice. Mice treated with hyperbaric oxygen were exposed BID to 100% oxygen at 303 kilopascals (kPa) pressure for 90 minutes. The total exposure time to HBO for surviving animals was nine hours. HBO alone did not enhance survivability of saline-injected mice (controls) when compared to mice exposed to air at ambient pressure. Survival of infected mice treated with either clindamycin or metronidazole was significantly longer than groups treated with penicillin or imipenem ($P < 0.05$). HBO alone or in combination with the four antimicrobial agents evaluated did not statistically improve survival of mice infected with a lethal dose of C. perfringens.

INTRODUCTION

Therapy for gas gangrene combines three different modalities: appropriate surgical debridement, antimicrobial chemotherapy, and hyperbaric oxygen administration (1-7). The sodium salt of penicillin is currently the antimicrobial agent of choice for both chemoprophylaxis and treatment of gas gangrene in humans (8). Recent in vivo studies have demonstrated several antibiotics to be more efficacious than penicillin in treating gas gangrene in mouse models (9-12). Stevens et al. (9) showed that monotherapy with clindamycin, metronidazole, rifampin, and tetracycline significantly improved survival of C. perfringens (ATCC 13124) infected mice when compared to penicillin. In a similar mouse model, Traub (11) demonstrated that penicillin was ineffective against the same standard strain of C. perfringens, as well as five clinical isolates.

Newer antimicrobial agents have not been tested in combination with HBO in animal models of gas gangrene. In vitro and in vivo experiments have demonstrated that HBO alone is bacteriostatic or bactericidal for C. perfringens depending upon the dose of HBO used (13-15). Bactericidal effects were achieved in vitro against C. perfringens when cultures were treated with higher doses of HBO than could be tolerated by human patients (13). In 1965, Van Unnik showed that in vitro treatment of C. perfringens with a dose of HBO tolerated by human patients (100% oxygen, 3 ATA, 90

minutes), had no effect on growth or survival of the bacteria (16). However, alpha toxin production was inhibited by the same dose of HBO (16).

The purpose of the current study was to test the efficacy of HBO alone and in combination with antimicrobial agents in preventing mortality in a mouse model of gas gangrene. To this end, mice injected with a lethal dose of C. perfringens were treated with HBO alone or in combination with penicillin, imipenem, clindamycin, or metronidazole.

MATERIALS AND METHODS

Animals. Two hundred female CD1 mice (15-20 grams) were obtained from Charles River Laboratories, Wilmington, DE for use in these experiments. Female mice were used because they tend to fight less in a restricted environment. The mice were maintained on standard laboratory chow and water ad libitum, except during HBO treatments when water was removed. Mice were housed under the following conditions: room temperature, 12-hour light-dark cycles, and 50-70% humidity. During experiments the mice were kept in 29 X 19 X 13 cm polystyrene cages (6 mice/cage). The experiments reported herein were conducted according to the principles described in the Guide for the Care and Use of Laboratory Animals published by the U.S. Department of Health and Human Services. The experiments were approved by the Laboratory Animal Care and Use Committee of the Armed Forces Institute of Pathology, Washington, DC.

Pathogenic Organism. Clostridium perfringens type A (strain ATCC 13124) was used in the study (American Type Culture Collection Rockville, MD). Organisms were maintained anaerobically in cooked meat media with hemin and vitamin K (Remel, Lenexa, KS) at room temperature. Organisms were recultured every two weeks. Prior to each experiment, bacteria were cultured in aerobic and anaerobic conditions on brucella agar plates with 5% sheep blood (Remel) to

insure a pure culture. Anaerobic conditions were created using the GasPak system (Becton Dickinson Microbiology Systems, Cockeysville, MD). Bacteria were scraped from plates with a loop, inoculated into Brewer thioglycollate broth (Difco Laboratories, Detroit, MI), and incubated overnight at 37°C. After incubation, the organisms were centrifuged at 2,500 g for 15 minutes and then resuspended in 10 ml of sterile normal saline. Bacterial concentration of the suspension was estimated using the optical density at 650 nm. Ten-fold dilutions of the bacterial suspension were made in sterile saline and 0.1 ml of the 10^{-7} and 10^{-8} dilutions were spread on blood agar plates. After 24 hour incubation under anaerobic conditions, the number of colony-forming units (CFU) was determined. The concentration of C. perfringens used for injection was at least 1×10^{10} CFU/ml, to insure that 0.1 ml injected into mice yielded greater than 10^9 CFU/ml.

Experimental Design. Two hundred mice were randomized for antibiotic treatment and oxygen exposure. Each treatment group (8) and each control group (2) consisted of 20 mice injected with the same dose of viable C. perfringens. Mice were injected i.p. with a single antibiotic at 0.5 and 8 hours postinoculation on day 1 of each experiment. Antibiotic doses given were: clindamycin (8.6 mg/kg), imipenem (30 mg/kg), penicillin G (380 mg/kg), and metronidazole (75 mg/kg). BID antibiotic treatment was also given on days 2 and 3 of each experiment. Immediately

following each antibiotic treatment, animals randomized to the HBO groups received a 90 minute exposure to 100% oxygen at 303 kPa pressure. The remaining groups of animals were treated with air at ambient pressure. Either HBO or air at ambient pressure was administered to mice for nine hours over a three day period. Mice were monitored for 72 hours and time of death was recorded.

C. perfringens Inoculation. The highly reproducible and lethal mouse model of gas gangrene as described by Stevens et al. (9,10) was used in this study. Briefly, $> 10^9$ CFU of Clostridium perfringens in a volume of 0.1 ml was injected into the right upper thigh muscles of mice through a 26 guage needle. Swollen, hemorrhagic thighs with crepitus and tissue necrosis were seen in infected mice. Injection of $> 10^9$ CFU of C. perfringens is uniformly lethal in saline-treated control animals within 24 hours.

Antimicrobial Agents. Penicillin G, clindamycin, and metronidazole (Sigma Chemical Company, St. Louis, MO) and imipenem (Merck, Sharp and Dohme, Rahway, NJ) were used in the study. All antibiotics were solublized in deionized water and sterilized by passage through a 0.2 um membrane filter (Nalge Sybron Corp., Rochester, NY). All antibiotics were administered i.p. in a volume of 0.3 ml per dose immediately after C. perfringens inoculation; control mice received 0.3 ml sterile deionized water.

Hyperbaric Oxygen Treatment. Treatments were given in small (38 cm in diameter, 76 cm in length) cylindrical metal chambers constructed by the Division of Altitude and Hyperbaric Physiology at the Armed Forces Institute of Pathology. Mice in hyperbaric oxygen treatment groups were placed in a hyperbaric chamber which was flushed with 100% oxygen before compression. The maximum number of mice placed in each chamber at one time was 12. Mice were compressed and decompressed at a rate of 3.07 kPa/ 2 sec (equivalent to 1 foot of sea water/2 sec). These descent/ascent rates are similar to those used for human patients. Animals were observed during pressurization and upon reaching the desired treatment pressure (303 kPa). Continuous ventilation was used in order to stabilize the gas composition and temperature in the chamber. Mice were exposed to 100% oxygen at a pressure of 303 kPa for 90 minutes. Control mice were placed in identical chambers for the same 90 minute period and were ventilated with air, but were not pressurized.

Statistical Analysis. Two nonparametric methods were used to compare survival curves. The test statistics used for testing equal survival curves were Gehan's Generalized Wilcoxon test (17,18) and Logrank test (19). The level of significance was chosen at $P < 0.05$.

RESULTS

Course of Experimental Infection. Experimentally infected CD-1 mice were moribund with piloerection within 4 hours of injection of $>10^9$ washed C. perfringens. Preliminary experiments confirmed that injection of $<10^9$ C. perfringens does not produce disease in this model of clostridial myonecrosis. As the disease progressed, mice exhibited swollen, hemorrhagic right thighs with cyanosis of the hindfoot and loss of limb function. Gross hematuria was noted in all animals which died.

More than 90% of untreated mice (controls) were dead within 8 hours of bacterial injection (Figs. 1 and 2). Preliminary experiments showed that a two hour delay in antibiotic treatment after bacterial injection shortened the time to death by two hours. At necropsy, impression smears of dissected right thigh muscle revealed large gram-positive rods. Cultures of infected muscle yielded hemolytic colonies of C. perfringens on Brucella blood agar plates.

Influence of Antimicrobials and HBO on Survival of Infected Mice
As shown in Figs. 1 and 2, HBO treatment alone did not significantly enhance survivability in saline-treated mice when compared to controls ($P<0.05$). Survival in all groups treated with antimicrobial agents was significantly greater than saline-injected controls ($P<0.05$). Survival in groups treated with

clindamycin (17 mg/kg/day) was significantly greater than those groups treated with penicillin G (780 mg/kg/day) [Fig. 1] or imipenem (comparison not shown). A trend toward increased mortality was seen in mice treated with clindamycin and HBO in combination, but this observation was not statistically significant. HBO did not influence the survival of penicillin G-treated mice as compared to air. Groups of mice treated with metronidazole (150 mg/kg/day) survived significantly longer than mice treated with imipenem (60 mg/kg/day) [Fig. 2] or penicillin (comparison not shown). At eight hours, a significant difference in the number survivors was seen between mice treated with imipenem in air (85%) and those treated with the combination of imipenem and HBO (20%). No difference was seen between the imipenem groups (air vs. HBO) by 12 hours, as all mice had perished by that time. In a similar pattern to that seen in clindamycin treated mice, the survivability for mice treated with metronidazole and HBO tended to be worse than for those treated in air. However, no statistical significance was found. No differences were in survival were observed between groups of mice treated with clindamycin or metronidazole (comparison not shown).

DISCUSSION

In the current study, HBO alone did not significantly alter the survival of saline-injected mice, even though HBO therapy was initiated shortly following the injection of pathogenic clostridia. Our experiments employed a lethal model of gas gangrene to observe the combined effects of HBO and antibiotics since untreated human gas gangrene infections are lethal. As such, our observations are in agreement with the study of Demello et al. which showed that HBO used alone did not prevent mortality in a lethal model of gas gangrene in dogs (20). The HBO treatment regimen used in their study was similar to that used for human patients: three HBO treatments (100% O₂ at 3 ATA) during the first 24 hours and a total duration of HBO exposure of 12 hours over three days. Although their HBO treatment protocol was more intensive than ours, HBO still did not prevent mortality.

In contrast, studies of other investigators showed a marked benefit of HBO in semi-lethal mouse models of gas gangrene in which mortality was only 50-60% in infected control animals (14,15). In both studies, mice were given five HBO treatments (100% O₂, 3 ATA, 90 minutes) within the first 26 to 27 hours postinoculation. Such intensive HBO treatment protocols would exceed the toxicity limits for oxygen in human patients (21). When less intensive HBO protocols were used, no significant difference in survival was seen between control and HBO-exposed

animals (14,15).

At the doses of antimicrobials used in this study, groups of mice treated with clindamycin and metronidazole survived significantly longer than groups of mice treated with either penicillin G or imipenem. These observations are in agreement with the work of Stevens et al. (9) and Traub (11). In contrast to work done by Traub, imipenem did not prolong survival of mice in our study. The same daily dose of imipenem (60 mg) reduced mortality in a similar mouse model with the same strain of C. perfringens (11). The difference in these results was probably due to an inoculum effect. The inoculum used to infect mice was approximately $1.5 \log_{10}$ CFU less than the inoculum used in our study.

HBO did not potentiate the efficacy of any antimicrobials tested in this study. In dogs treated with HBO and penicillin, one additional animal survived (55%) when compared to penicillin treatment alone. Demello demonstrated that 50% of the dogs with gas gangrene survived when treated with penicillin alone (20). HBO, alone or combined with either surgery or penicillin had no effect on survival of dogs with gas gangrene. Yet, 95% of the dogs treated with triple therapy (surgery, penicillin, HBO) survived. Two clinical reports (23,24) have compared triple therapy with antibiotics plus surgery in patients with gas gangrene. Although the numbers of patients described in these

reports were small, mortality was decreased in HBO-treated patients. Thus, in contrast to our findings in mice, the experiments described by Demello combined with the clinical reports provide support for the use of HBO as an adjunctive treatment for gas gangrene.

The results our study in a mouse model show that HBO does not potentiate any of the antimicrobial agents with proven efficacy against C. perfringens in vivo, including the drug of choice, penicillin. We speculate that in a model of gas gangrene where triple therapy was used, the surgical removal of necrotic tissue may promote the delivery of antimicrobial agents and oxygen to infected tissues. Protein synthesis inhibitors, such as clindamycin, could stop alpha toxin production in clostridia. In surgically-opened gangrenous tissues, HBO treatment may elevate PO_2 s to levels which could inhibit alpha toxin production. C. perfringens alpha toxin contains at least four biological activities: hemolytic, necrotic, lethal, and phospholipase C (24). It is thought that any or all of the biological activities of C. perfringens alpha toxin may contribute to the pathogenesis of gas gangrene. Inactivation of this major virulence factor by HBO and/or antibiotic which inhibit protein synthesis may prevent the spread of the clostridial infection to healthy tissues.

In summary, our data demonstrate that HBO alone does not enhance survival of mice with gas gangrene nor does HBO

potentiate the anti-clostridial activity of any of the antimicrobial agents tested. Future studies should be designed to examine the combined effects of HBO and antimicrobial agents on alpha toxin production by clostridia.

REFERENCES

1. Hart GB, Lamb RC, Strauss MB: Gas gangrene: I a collective review. J Trauma 23:991, 1983
2. Hedstrom SA: Differential diagnosis and treatment of gas-producing infections. Act Chir Scand 141:582, 1975
3. Guidi ML, Proietti R, Carducci P, et al.: The combined use of hyperbaric oxygen, antibiotics and surgery in the treatment of gas gangrene. Resuscitation 9:267, 1981
4. Bakker DJ: The treatment of gas gangrene with hyperbaric oxygen - results of 15 years experience. In Smith G (ed) Proceedings of the sixth international congress on hyperbaric medicine Scotland University Press, Aberdeen, 295, 1979
5. Bakker DJ: The use of hyperbaric oxygen in the treatment of certain infectious diseases especially gas gangrene and acute dermal gangrene. Drukkerijveenman BV, Wageningen, 1984
6. Kofoed H, Riegels-Nielsen P: Myonecrotic gas gangrene of the extremities. Acta Orthop Scand 54:220, 1983

7. Unsworth IP, Sharp PA: Gas gangrene. An 11-year review of 73 cases managed with hyperbaric oxygen. Med J Aust 140:256, 1984
8. Hart GB, Strauss MB: Gas gangrene - clostridial myonecrosis: a review. J Hyperbar Med 5:125, 1990
9. Stevens DL, Maier KA, Laine BM, et al.: Comparison of clindamycin, rifampin, tetracycline, metronidazole and penicillin for efficacy in prevention of experimental gas gangrene due to Clostridium perfringens. J Infect Dis 155:220, 1987
10. Stevens DL, Laine BM, Mitten JE: Comparison of single and combination antimicrobial agents for prevention of experimental gas gangrene caused by Clostridium perfringens. Antimicrob Agents Chemother 31:312, 1987
11. Traub WH: Chemotherapy of experimental (murine) Clostridium perfringens Type A gas gangrene. Chemotherapy 34:472, 1988
12. Traub WH: Clostridium perfringens Type A. Comparison of in vitro and in vivo activity of twelve antimicrobial drugs. Chemotherapy 32:59, 1986

13. Hill GB, Osterhout S: Experimental effects of hyperbaric oxygen on selected clostridial species: I In-vitro studies. J Infect Dis 125:17, 1972
14. Hill GB, Osterhout S: Experimental effects of hyperbaric oxygen on selected clostridial species: II In-vivo studies in mice. J Infect Dis 125:26, 1972.
15. Holland JA, Hill GB, Wolfe WG et al.: Experimental and clinical experience with hyperbaric oxygen in the treatment of clostridial myonecrosis. Surgery 77:75, 1975
16. Van Unnik, AJM: Inhibition of toxin production in Clostridium perfringens in vitro by hyperbaric oxygen. Antonie van Leeuwenhoek 31:181, 1965
17. Gehan, EA: A generalized Wilcoxon test for comparing arbitrarily singly-censored samples. Biometrika 52:203, 1965
18. Gehan EA: A generalized two-sample Wilcoxon test for doubly-censored data. Biometrika 52:650, 1965
19. Peto R, Peto J: Asymptotically efficient rank in variant procedures. J Royal Statistical Soc 135:185, 1972

20. Demello FJ, Haglin JJ, Hitchcock CR: Comparative study of experimental Clostridium perfringens infection in dogs treated with antibiotics, surgery and hyperbaric oxygen. Surgery 73:936, 1973
21. Wright WB: Use of the University of Pennsylvania, Institute for Environmental Medicine procedure for calculation of cumulative pulmonary oxygen toxicity. U.S. Navy Experimental Diving Unit Report NEDU 2-72.
22. Jackson RW, Waddell JP: Hyperbaric oxygen in the management of clostridial myonecrosis (gas gangrene). Clin Orthop Relat Res 96:271, 1973.
23. Schweigel JF, Shim SS: A comparison of the treatment of gas gangrene with and without hyperbaric oxygen. Surg Gynecol Obstet 136:969, 1973
24. Sakurai J, Fujii Y, Torii K et al.: Dissociation of various biological activities of Clostridium perfringens alpha toxin by chemical modification. Toxicon 27:317, 1989

ACKNOWLEDGEMENTS

This study was supported by grant 90-0317 from the Air Force Office of Scientific Research. Kenneth Muhvich was supported by the Callender-Binford Fellowship in pathology sponsored by the American Registry of Pathology, Washington, D.C. 20306-6000.

We thank David Nelson for excellent technical support and maintenance of the experimental hyperbaric chambers. We also thank Patricia Schleiff for statistical support and Darrell Criswell for helpful discussions.

Figure Legends

Fig. 1. Survival curves for CD-1 mice injected i.m. with $>10^9$ C. perfringens ATCC 13124. Each curve represents mice (n = 20) treated BID with air at ambient pressure (closed symbols) or hyperbaric oxygen [100% O₂ at 303 kPa for 90 minutes] (open symbols) with or without antimicrobial chemotherapy.

Fig. 2. Survival curves for CD-1 mice injected i.m. with $>10^9$ C. perfringens ATCC 13124. Each curve represents mice (n = 20) treated BID with air at ambient pressure (closed symbols) or HBO (open symbols) with or without antimicrobial chemotherapy.

